THE THIRD SERUM COMPONENT.*

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IN previous papers[1] it has been pointed out that heated hemolytic serum is so changed by exposure to blood corpuscles that direct quantitative comparisons between it and unexposed sera are impossible. This finding is of fundamental importance in serum pathology, as the assumption of the direct quantitative comparability of different sera, toxins, antitoxins, and the like is the basis for all quantitative conclusions thus far drawn in the subject. Work was therefore undertaken to determine the nature of this change, and to discover, if possible, means of overcoming it, and of thus making analyses possible.

This work has led to the conclusion that, in addition to the complement and amboceptor currently assumed as the only active components of hemolytic serum, there is a third component, or series of components, having a marked influence on hemolytic power. Preliminary reports on this third component have already been made.[2] Although the work is as yet incomplete, it is thought best, at this time, to bring together the data thus far gathered, in order to point the way to the future developments of the subject.

There are two ways in which it is conceivable that exposure of heated hemolytic serum to blood corpuscles may produce qualitative changes in that serum. First, such exposure may change the chemical nature of certain individual serum components. Second, unequal absorption of the different components by the blood corpuscles may bring about disturbances in the quantitative relations, and thus cause a qualitative change in the serum as a whole.

According to current theories there are present in such sera two components: (1) a thermostable amboceptor; and (2) the third

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[1] *Jour. Inject. Dis.*, 1905, 2, pp. 485–97; *Trans. Chicago Path. Soc.*, 1905, 6, pp. 319–24; *Jour. of Biol. Chem.*, 1906, 1, pp. 213–18; *Centralbl. f. Bakt.*, 1906, 40, pp. 386–88.

[2] *Trans. Chicago Path. Soc.*, 1905, 6, pp. 351–58; *Science*, 1906, 23, p. 209; *Jour. Infect. Dis.*, 1906, 3, pp. 225–77.

component, which consists of a mixture of unchanged elements of normal serum, degeneration products of complement ("complementoid"), and hypothetical split products of other thermolabile serum elements. Changes in individual components could not be tested for experimentally till the possibility of there being important changes in quantitative relations is ruled out. Experiments were therefore planned to test the effect on hemolytic power of altering the relative amounts of amboceptor and third component.

To do this various mixtures of amboceptor[1] (heated hemolytic serum containing, of course, its own volume of third component) and third component (heated normal serum) were made, and amboceptor curves[2] plotted with these mixtures. Two of the simplest curves thus obtained are shown in Fig. 1. Here Curve A represents the changes in hemolytic power as increasing amounts of pure amboceptor are added to a constant amount of complement (normal serum). Curve B shows the changes as increasing amounts of the same amboceptor, plus an equal volume of third component, are added to the same amount of complement. Both curves, of course, are plotted to the same scale, and were made with the same sera, the same corpuscles, and on the same day.

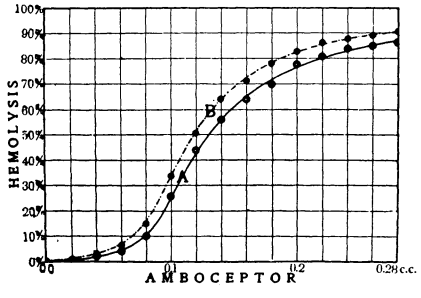

FIG. 1.—Effect of third component on amboceptor curve. A=curve showing changes in hemolytic power as increasing amounts of amboceptor are added to a constant amount of complement (0.25 c.c.). B=Curve showing changes as increasing amounts of the same amboceptor, plus an equal volume of third component, are so added. Curves made with the same sera, same corpuscles, and on the same day.

From these curves it is seen that the addition of third component causes, under the conditions of this particular experiment, an increase in hemolytic power. Less evident is the fact that it also causes a qualitative change in that power, the two sera no longer being analytically comparable.

This qualitative change is most evident if attempts are made to estimate the strength of the new amboceptor in terms of the old. Such an estimation is shown in Table 1, from which

[1] For technic, material, etc., see *Jour. Infect. Dis.*, 1905, 2, p. 461.

[2] *Ibid.*, 2, p. 471; *Centralbl. f. Bakt.*, 1906, 40, p. 401.

it is seen that the new amboceptor is apparently anywhere from 108 per cent to 120 per cent the strength of the original amboceptor, the percentage depending on the amounts taken for comparison.

TABLE 1.

EFFECT OF THIRD COMPONENT ON APPARENT STRENGTH OF AMBOCEPTOR.

(DATA FROM FIG. 1.)

HEMOLYSIS	AMBOCEPTOR	AMBOCEPTOR PLUS EQUAL VOLUME OF THIRD COMPONENT	APPARENT STRENGTH	
			Equal Amounts of Amboceptor Compared	Equal Volumes of Total Serum Compared
10%	0.78 c.c.	0.68 c.c.	115%	57.5%
20	0.94	0.84	112	56.0
30	1.04	0.96	108	54.0
40	1.16	1.06	109	54.5
50	1.30	1.18	110	55.0
60	1.48	1.32	112	56.0
70	1.74	1.52	115	57.5
80	2.20	1.86	118	59.0
87	2.80	2.34	120	60.0
		Real percentage of amboceptor, 100%		50.0%

The same phenomenon is seen, in more striking form, in Figs. 2 and 3. The corresponding calculated apparent strengths of amboceptor are shown in Tables 2 and 3.

TABLE 2.

EFFECT OF THIRD COMPONENT ON APPARENT STRENGTH OF AMBOCEPTOR.

(DATA FROM FIG. 2.)

HEMOLYSIS	AMBOCEPTOR	AMBOCEPTOR PLUS THIRD COMPONENT			APPARENT STRENGTH					
		Half Volume	Equal Volume	Twice Volume	Equal Amounts of Amboceptor Compared			Equal Volumes of Total Serum Compared		
		Curve B	Curve C	Curve D	B	C	D	B	C	D
	c.c.	c.c.	c.c.	c.c.						
10%	0.072	0.064	0.060	0.052	113%	120%	139%	75%	60.0%	45%
20	0.092	0.087	0.083	0.078	105	110	117	70	55.0	39
30	0.102	0.099	0.096	0.091	103	106	112	69	53.0	38
40	0.108	0.106	0.103	0.099	102	105	109	68	52.5	36
50	0.116	0.113	0.110	0.105	103	106	110	69	53.0	37
60	0.122	0.119	0.116	0.111	102	105	110	68	52.5	37
70	0.131	0.127	0.123	0.117	103	107	112	69	53.5	37
80	0.145	0.138	0.132	0.125	105	110	116	70	55.0	39
90	0.176	0.160	0.150	0.140	110	117	126	73	58.5	42
100	0.240	0.208	0.187	0.166	115	128	145	77	64.0	48
		Real percentage of amboceptor,			100%	100%	100%	66⅔%	50.0%	33⅓%

TABLE 3.

EFFECT OF THIRD COMPONENT ON APPARENT STRENGTH OF AMBOCEPTOR.

(DATA FROM FIG. 3.)

HEMOLYSIS	AMBOCEPTOR	AMBOCEPTOR PLUS THIRD COMPONENT			APPARENT STRENGTH					
		Twice Volume	Seven Times Volume	Seventeen Times Volume	Equal Amounts of Amboceptor Compared			Equal Volumes of Tota Serum Compared		
		Curve B	Curve C	Curve D	B	C	D	B	C	D
	c.c.	c.c.	c.c.	c.c.						
10%	0.346	0.296	0.256	0.196	116%	135%	177%	39%	17%	9.8%
20	0.408	0.358	0.330	0.288	114	124	142	38	15	7.9
30	0.452	0.408	0.380	0.338	111	119	133	37	15	7.3
40	0.492	0.456	0.428	0.384	108	115	128	36	14	7.1
50	0.534	0.508	0.476	0.436	105	112	122	35	14	6.8
60	0.576	0.556	0.528	0.488	103	109	118	34	14	6.5
70	0.618	0.608	0.576	0.544	102	107	114	34	13	6.3
80	0.664	0.656	0.626	0.616	101	106	108	34	13	6.0
90	0.734	0.720	0.692	0.744	102	106	98	34	13	5.4
100	0.812	0.800	0.788	0.888	101	103	91	34	13	5.0
		Real percentage of amboceptor,			100%	100%	100%	33⅓%	12.5%	5.6%

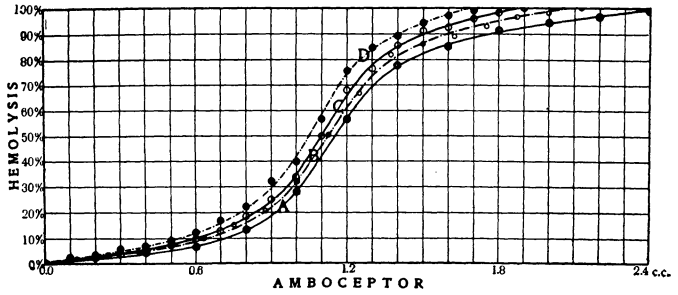

FIG. 2.—Effect of third component on amboceptor curve. A=Curve, as in Fig. 1, showing changes in hemolytic power as increasing amounts of amboceptor are added to a constant amount of complement (0.20 c.c.). B=Curve showing changes as increasing amounts of the same amboceptor, plus half its volume of third component, are so added. C=Curve with an equal volume of third component. D=Curve with two times its volume of third component.

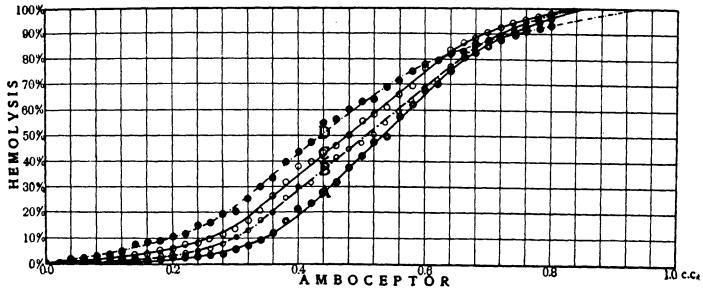

FIG. 3.—Effect of third component on amboceptor curve. A=Curve, as in Figs. 1 and 2, showing changes in hemolytic power as increasing amounts of amboceptor are added to a constant amount of complement (0.22 c.c.). B=Curve showing changes as increasing amounts of the same amboceptor, plus twice its volume of third component, are so added. C=Curve with seven times its volume of third component. D=Curve with 17 times its volume of third component.

In Curve D, Fig. 3, the third component is seen to possess a double power, that of increasing hemolysis when present in comparatively small amounts (maximum 177 per cent), but of decreasing it when present in larger amounts (minimum 91 per cent). This curve strikingly illustrates the fact that changing the relative amounts of amboceptor and third component changes the serum so that it liberates hemoglobin according to a new quantitative law. This change probably accounts for part at least of the change observed in heated hemolytic serum after exposure to corpuscles, as it is inconceivable that all of the serum components could be absorbed equally by the corpuscles. Whether it accounts for the total change in such exposed serum, or not, is still under investigation.

The discovery that under most conditions the third component possesses auxilytic properties (*αὔξειν*, to increase) is so at variance with the current belief that "complementoid" lessens serum action by forming inactive amboceptor-"complementoid" compounds, that work was planned to determine, with greater accuracy, the exact rôle of third complement in hemolytic action. To do this, increasing amounts of third component were added to a constant amount of hemolytic serum, and the resulting hemolyses plotted as a curve, showing the changes in hemolytic power as the third component increases.

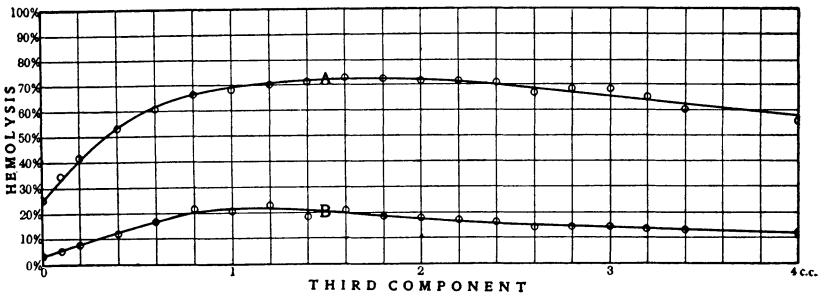

FIG. 4.—Third component curves. Curves showing changes in hemolytic power as increasing amounts of third component are added to constant amounts of hemolytic serum. A = Curve with a constant amount of hemolytic serum capable of producing 26 per cent hemolysis. B = Curve with a constant capable of producing 4 per cent. The curves show an auxilytic action of the third component.

Two curves, obtained in this way, are shown in Fig. 4, and a series of six curves, so obtained, in Fig. 5. Four additional curves are given in Fig. 6. Fig. 6 differs from Figs. 4 and 5, in that, in

place of a constant amount of hemolytic serum, a constant amount of an artificial, hemolytic, amboceptor-complement mixture was used.

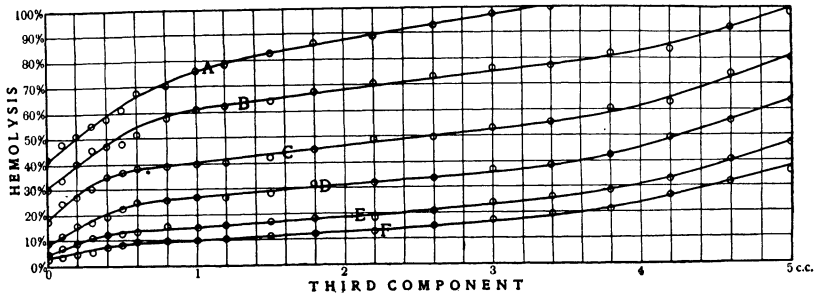

FIG. 5.—Third component curves. Curves, as in Fig. 4, showing changes in hemolytic power as increasing amounts of third component are added to constant amounts of hemolytic serum. The constant amounts of hemolytic serum used in this experiment are capable of producing: 42 per cent hemolysis in Curve A, 31 per cent in Curve B, 18 per cent in Curve C, 8 per cent in Curve D, 5 per cent in Curve E, and 3 per cent in Curve F. The curves show a purely auxilytic action of the third component.

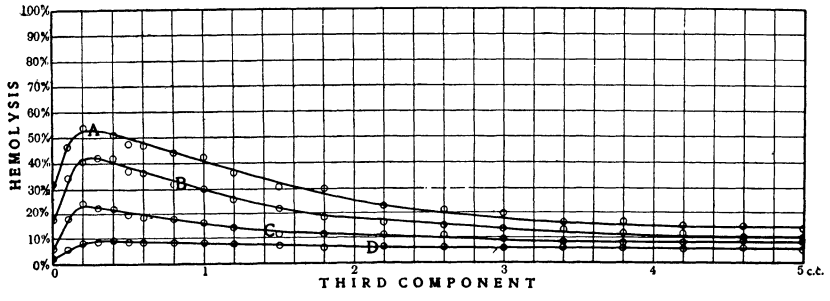

FIG. 6.—Third component curves. Curves, as in Figs. 4 and 5, showing changes in hemolytic power as increasing amounts of third component are added to constant amounts of hemolytic serum. The constant amounts of hemolytic serum used in this experiment are capable of producing: 30 per cent hemolysis in Curve A, 17 per cent in Curve B, 6 per cent in Curve C, and 2.5 per cent in Curve D. The curves show an auxilytic action of third component, when used in small quantities; but an antilytic action in larger amounts.

A comparison of these curves is best made by superposing sample curves of each set. Such a superposition is shown in Fig. 7 (Curves B, C, and D). To the figure there have been added four additional curves (A, E, F, and G), taken from subsequent experiments.

From Fig. 7 it is seen that the third component may possess at least four distinct actions: first, it may be practically without effect on hemolysis, as in Curve E; second, it may possess a strong antilytic or inhibiting action on hemolysis, as in Curve G; third, it may

possess a greater or less degree of auxilytic, or increasing action on hemolysis, as in Curves A, B, C, and F; and, fourth, it may pessess

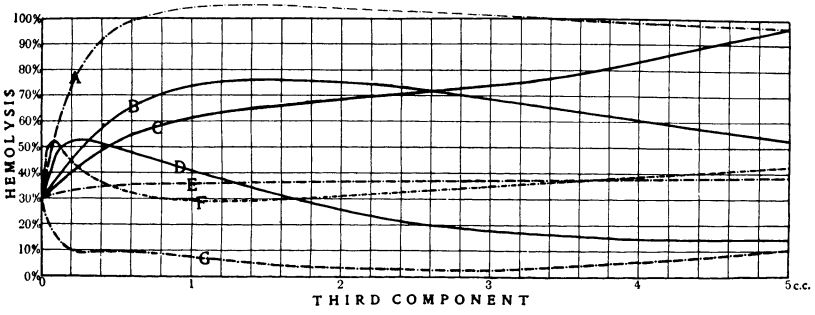

FIG. 7.—Third component curves. Sample curves superimposed from different experiments, showin four different types of third component action.

an auxilytic action when used in small quantities, but an antilytic action in larger amounts, as seen in Curve D. To what are these differences due?

There are two ways in which is it conceivable that the differences in the action of the third complement in different experiments may be brought about. First, it is possible that the sera of different normal animals may differ in the amount or kind of third component they contain. Second, there is the possibility that differences in the treatment of the same serum under different experimental conditions may produce differences in the third component.

To determine whether or not there are differences in the third components of different normal animals, blood was drawn in equal amounts from six normal goats, on the same afternoon, and the sera allowed to separate in the same ice-chest over night. The next forenoon, equal volumes of the sera were measured out into flasks of a uniform size, and the six flasks heated to 56° C., in a thermostatic water-bath, such as is used in physico-chemical research, under conditions that assured perfect uniformity of heating. At the end of 60 minutes the flasks were removed from the water-bath, and cooled by immersing in ice-water. Curves were then plotted with the resulting third components.

The six curves obtained in this way are shown in Fig. 8. Five additional curves, obtained in the same way, but with the third components heated to 59° C., for 60 minutes, are shown in Fig. 9.

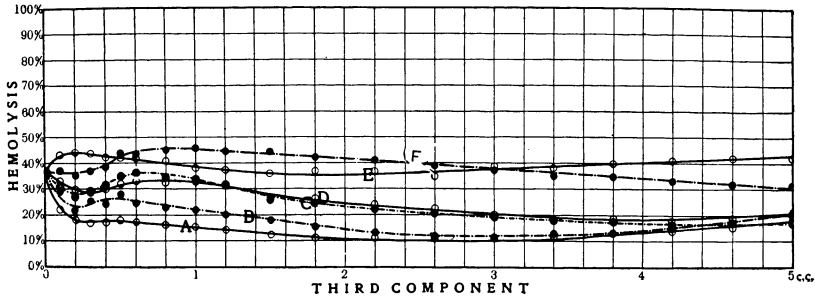

FIG. 8.—Comparison of third components of different normal sera. Curves showing changes in hemolytic power as increasing amounts of third component from six different normal animals are added to the same constant amount of hemolytic serum. Third components heated to 56° C. for 60 minutes.

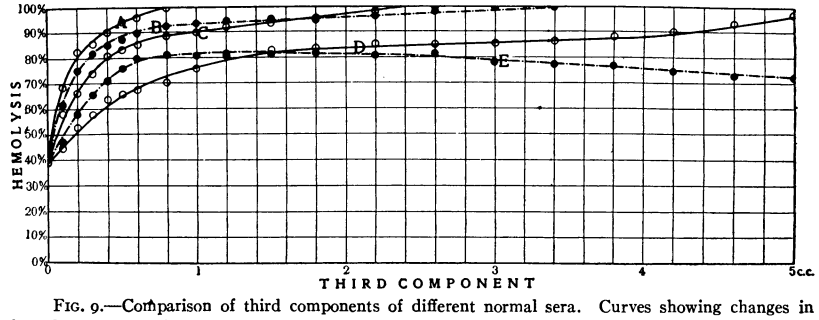

FIG. 9.—Comparison of third components of different normal sera. Curves showing changes in hemolytic power as increasing amounts of third component from five different normal animals are added to the same constant amounts of hemolytic serum. Third components heated to 59° C. for 60 minutes.

From Fig. 8 it is seen that no two of the six normal animals that were tested have sera that yield the same third component, under identical conditions. In four of the animals, the third component was to a greater or less degree antilytic (Curves A, B, C, and D), while in the other two it was, in certain amounts, slightly auxilytic (Curves E and F). In Fig. 9, while all the third components are strongly auxilytic, the differences between them are equally striking.

This finding is of broad biological and medical significance. Its main interest, however, in the present paper lies in its bearing on quantitative serum investigations. Quantitative measurements with hemolytic sera will be out of the question, till a method is devised by which the action of the third component can either be entirely eliminated, or, at least, made uniform in different sera. Two sera, possessing identical amboceptor and complement, may have widely

different hemolytic action, if the the third components of one is auxilytic, while that of the other is antilytic.

To test the second hypothesis, that differences in experimental conditions may produce differences in third component, serum was drawn in a large amount from one normal animal, or a large amount of carefully mixed sera from two or more animals was used, and equal volumes of this serum heated, at a uniform temperature, for different periods of time. Curves were then plotted with the resulting third components.

Five curves, obtained in this way, are shown in Fig. 10. Additional curves, so obtained, are given in Figs. 11 and 12.

From these curves it is seen that differences in the length of time of heating the serum may produce marked differences in the third component. In each set of curves, heating for more than 30 minutes

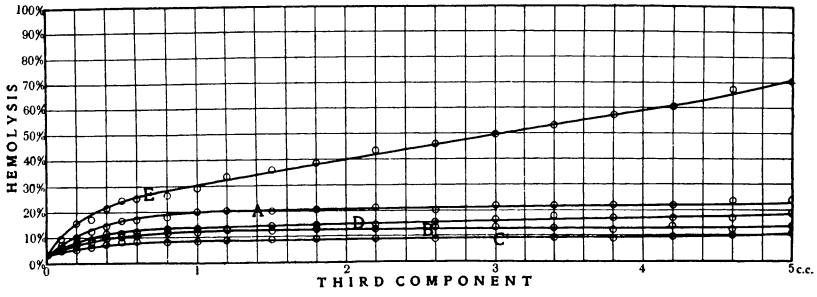

FIG. 10.—Effect of prolonged heating on the third component. Third component curves, as in Fig. 8, but with the same normal serum heated for different periods of time. A=Curve with serum heated to 56° C., for 30 minutes; B=Curve with serum heated 60 minutes; C=heated 100 minutes; D=150 minutes, and E=220 minutes.

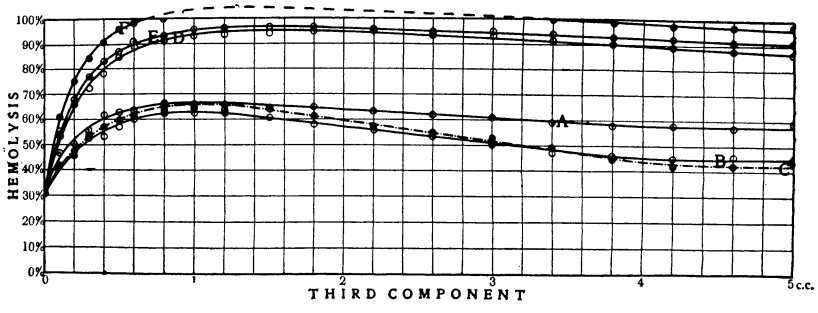

FIG. 11.—Effect of prolonged heating on the third component. Third component curves, as in Fig. 10, with the same normal serum heated to 56° for different periods of time. A=Curve with serum heated for 30 minutes; B, for 45 minutes; C, for 60 minutes; D, for 100 minutes; E for 130 minutes, and F, for 165 minutes.

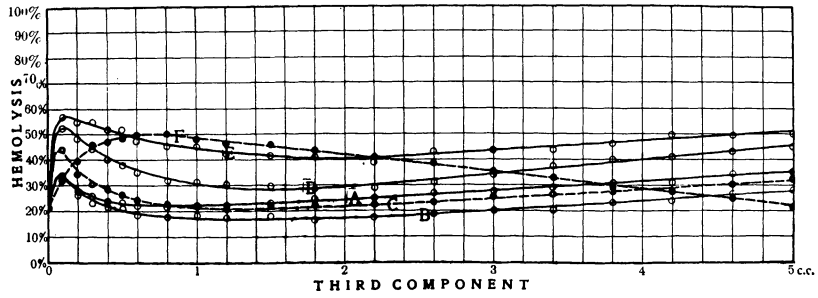

FIG. 12—Effect of prolonged heating on third component. Third component curves, as in Figs. 10 and 11, with the same normal serum, heated to 56° C., for different periods of time. Time of heating: Curve A=35 minutes, Curve B=65 minutes, Curve C=108 minutes, Curve D=165 minutes, Curve E =240 minutes, Curve F=330 minutes.

caused a distinct drop in the third component curve. This drop was succeeded by a marked rise when the serum was heated for more than 60 minutes. In Fig. 12, this rise was succeeded by a second fall and by a marked change in the nature of the curve, when the heating was prolonged beyond four hours.

In order to follow these changes in greater detail, a flask of normal serum was heated at a uniform temperature for 10 hours, and an accurately measured amount of serum removed, at 10-, 20-, and 30-minute intervals throughout that time, and tested for its effect on hemolytic power.

A curve obtained in this way, by heating the serum to 56° C., is shown in Fig. 13. Six curves of the same nature, obtained by using different constant amounts of third component, but the same

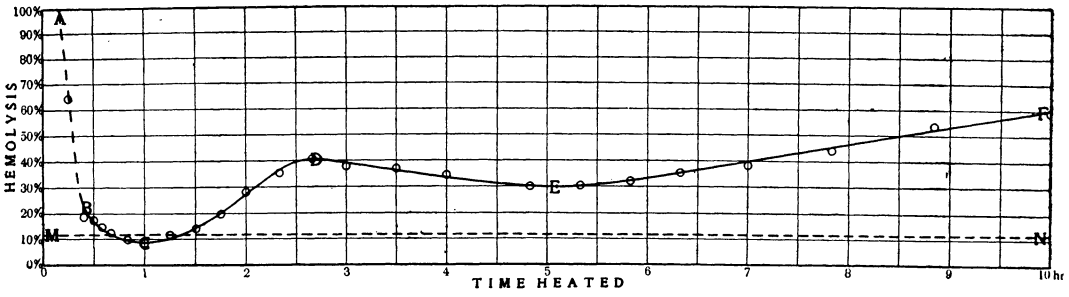

FIG. 13—Effect of prolonged heating on third component. Curve showing changes in auxilytic and antilytic properties of third component, when heated to 56° C. for 10 hours. Curve at each stage of the heating shows the effect on hemolytic power of adding a constant amount of third component (1.8 c.c.) to a constant amount of hemolytic serum. The hemolytic serum in itself, is capable of producing 12 per cent hemolysis (MN). Dotted portion of curve (AB)=curve before the complete destruction of complement. This curve is the average of the six curves shown in Fig. 14.

constant amount of hemolytic serum, are shown in Fig. 14. A curve obtained in the same way, but with serum heated 59° C., is shown in Fig. 15, and six parallel curves, so obtained, in Fig. 16.

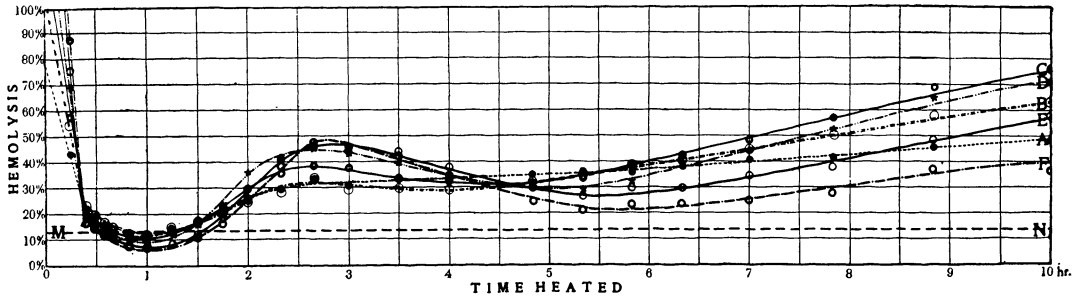

FIG. 14—Effect of prolonged heating on third component. Curve as in Fig. 13, showing changes in auxilytic and antilytic properties of third component, when heated to 56° C. for 10 hours. Constant amount of hemolytic serum used in this experiment is capable of producing 12 per cent hemolysis (MN). The constant amounts of third component used in each curve are: Curve A=0.25 c.c., Curve B=0.5 c.c., Curve C=1 c.c., Curve D=2 c.c., Curve E=3 c.c., and Curve F=4 c.c.

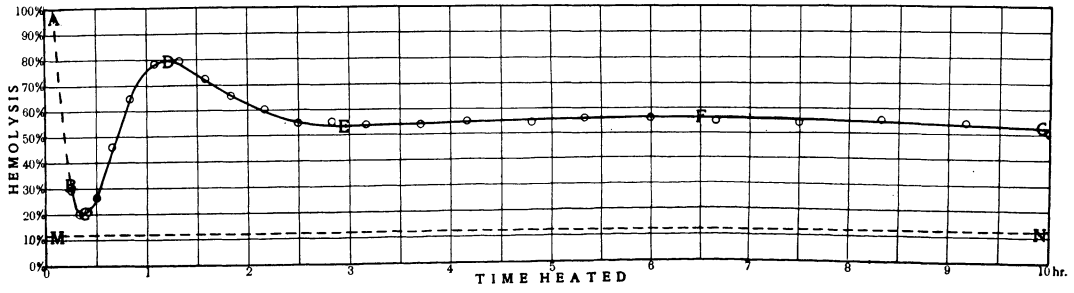

FIG. 15—Effect of prolonged heating on third component. Curve, as in Fig. 13, showing changes in auxilytic properties of third component, when heated to 59° C. for 10 hours. This curve is the average of the six curves in Fig. 16. Constant hemolytic serum is capable of producing 13 per cent hemolysis (MN). Dotted portion (AB) is curve before the complete destruction of complement.

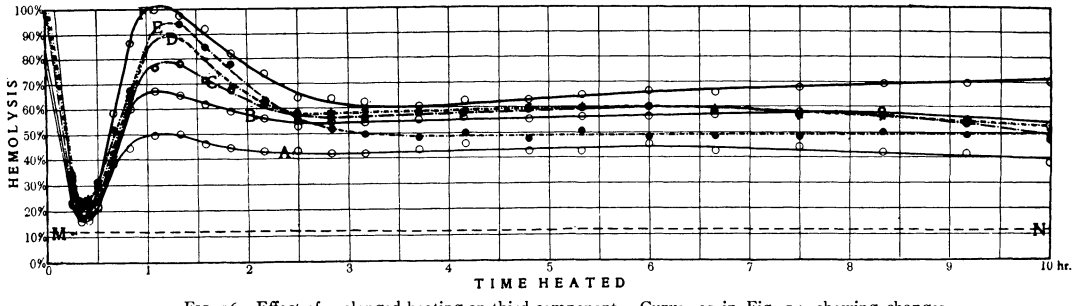

FIG. 16—Effect of prolonged heating on third component. Curve, as in Fig. 14, showing changes in auxilytic properties of third component when heated to 59° C. for 10 hours. The constant amounts of third component used in each curve are: Curve A=0.3 c.c., Curve B=0.6 c.c., Curve C=1 c.c., Curve D=1.5 c.c., Curve E=3 c.c., Curve F=5 c.c. Constant hemolytic seen capable of producing 13 per cent hemolysis (MN).

From these curves it is seen that, when serum is heated to 56° C., complete destruction of complement takes place in about 20 minutes. At that time the third component possesses a slight auxilytic action (primary auxilysin possibly due to a trace of undestroyed complement, though complement could not be detected experimentally). As the heating is continued beyond 20 minutes there is a diminution in this auxilytic power. The power completely disappears at the end of about 40 minutes' heating and is succeeded by an antilytic power. This antilytic action reaches a maximum in about 60 minutes, when it in turn decreases and entirely disappears in about 110 minutes.

On prolonging the heating beyond 110 minutes, there is produced a second auxilytic power (secondary auxilysin), which reaches a maximum in about three hours' heating. This secondary auxilysin in turn decreases, reaching a minimum at the end of about five hours. After this there is a gradual increase in auxilytic power (tertiary auxilysin), which apparently has not reached its maximum, at the end of 10 hours' heating.

When the serum is heated to 59° C., the same general phenomenon takes place, except that the production of the antilytic substance is then apparently masked by the more marked or more rapid production of the secondary auxilysin, and the destruction of the secondary auxilytic substance, is apparently modified by the more rapid production of the tertiary auxilysin. The tertiary auxilysin reaches a maximum in about six hours, after which there is a slight decrease in auxilytic power.

Changes in the nature of the third component curve during prolonged heating can be determined roughly from Figs. 14 and 16. Cross-sections of Fig. 14 at different periods of time give the third component curves shown in Fig. 17. Cross-sections of Fig. 16 give the curves shown in Fig. 18. Figs. 17 and 18 show the same general changes in the third component curve previously noted, except that here by taking the cross-sections at selected times, the observed changes are more pronounced.

It was shown in the fore part of the paper that there are differences between the third components of different normal animals, prepared under identical conditions. What is the cause of this difference?

It is conceivable that the difference is due either to differences in the amounts of the various auxilytic and antilytic substances

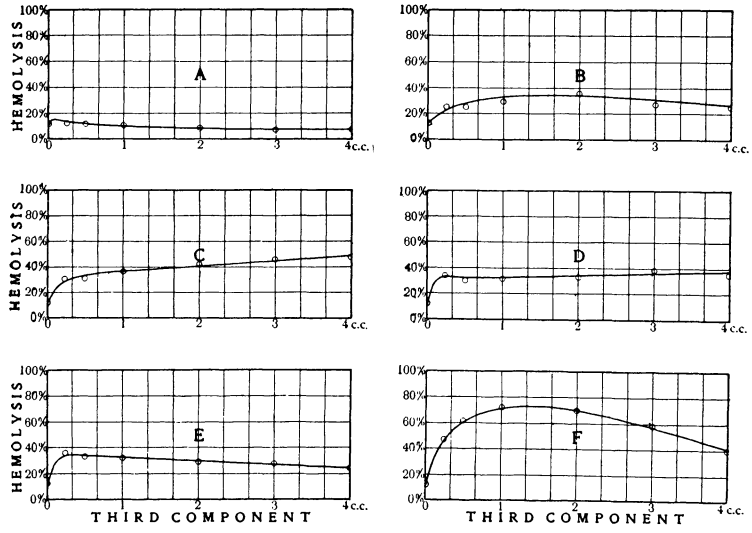

FIG. 17.—Changes in third component curve during prolonged heating. Curves drawn from data in Fig. 14. Length of heating: Curve A=60 minutes, Curve B=120 minutes, Curve C=180 minutes, Curve D=240 minutes, Curve E=300 minutes, Curve F=600 minutes. Temperature=56° C.

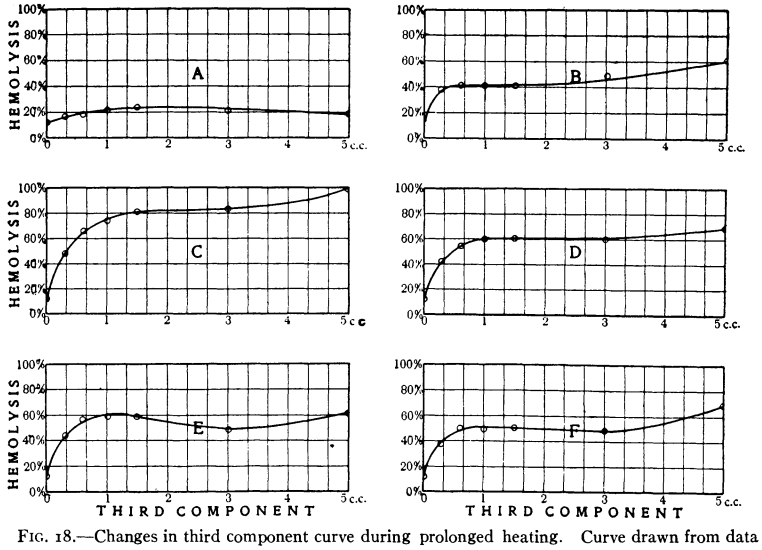

FIG. 18.—Changes in third component curve during prolonged heating. Curve drawn from data in Fig. 16. Length of heating: Curve A=20 minutes, Curve B=40 minutes, Curve C=60 minutes, Curve D=140 minutes, Curve E=240 minutes, Curve F=600 minutes. Temperature=59° C.

formed in these sera during prolonged heating, or to differences in the times at which these substances are so formed. To test these hypotheses, different normal sera were heated together in the same thermostatic water-bath, and parallel curves plotted, showing the changes in the auxilytic and antilytic properties of each serum during prolonged heating.

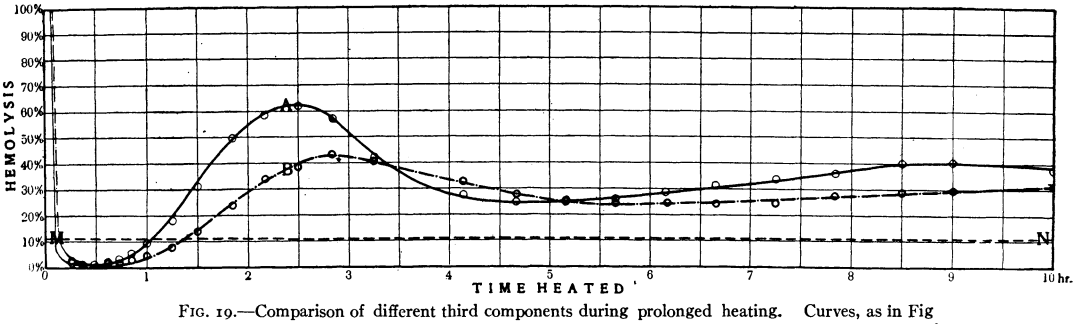

FIG. 19.—Comparison of different third components during prolonged heating. Curves, as in Fig 13, showing changes in auxilytic and antilytic properties of the third components of two different normal animals, when the sera are heated to 56° C. for 10 hours. The constant amount of hemolytic serum used in the experiment is capable of producing 11 per cent hemolysis (MN). Curve A is the average of Curves C and D of Fig. 20, and Curve B, the average of Curves A and B of the same figure.

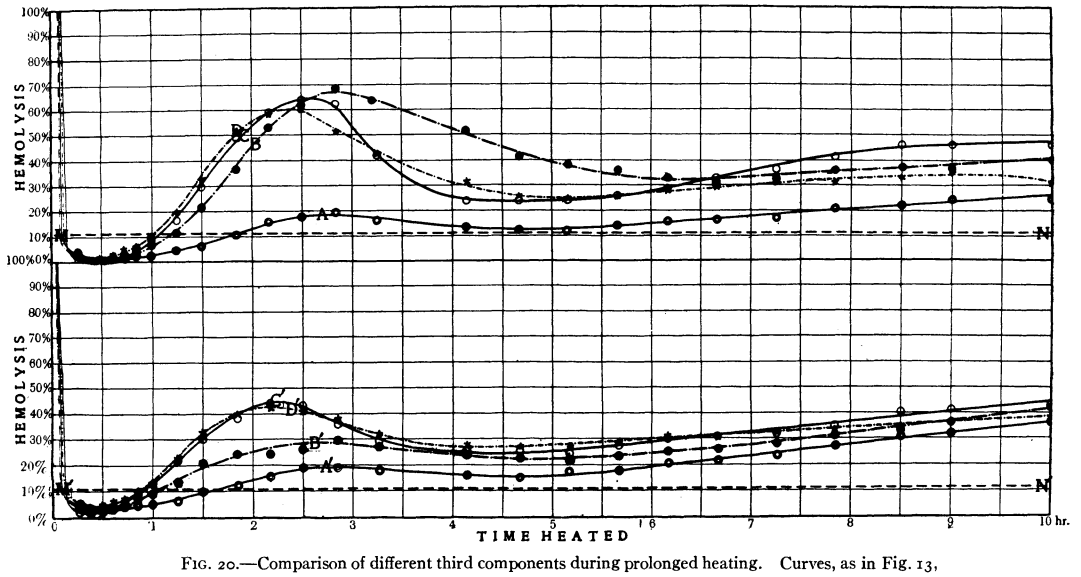

FIG. 20.—Comparison of different third components during prolonged heating. Curves, as in Fig. 13, showing changes in auxilytic and antilytic properties of the third components of the four different normal animals, when their sera are heated to 56° C. for 10 hours. The constant amount of third component used in the upper set of curves (A, B, C, and D)=3 c.c., in the lower set (A,′ B,′ C,′ and D′)=0.5 c.c. Constant hemolytic serum used in both sets is capable of producing 11 per cent hemolysis (MN, M′N′).

Two curves, obtained in this way, are shown in Fig. 19. Two sets of four curves each, so obtained, are given in Fig. 20.

From Figs. 19 and 20 it is seen that the difference observed between the third components of different normal sera, is due partly to slight differences in the times at which the various auxilytic and antilytic substances are produced or destroyed; but mainly due to differences in the amounts of these substances so produced. Thus, Curve A, of Fig. 20, shows the production of a very small amount of secondary auxilysin, while Curves B, C, and D show comparatively large amounts of that substance.

It should be noted, in concluding the presentation of this data, that at no time in any of the above experiments was the third component found to possess independent hemolytic powers, and at no time was it capable of sensitizing corpuscles or of reactivating amboceptor. The auxilysins have, in all cases, been incapable of acting, except in the presence of both amboceptor and complement.

No theory is as yet advanced as to the nature of the various auxilytic and antilytic substances herein reported, nor as to the bearing of this report on the fundamental concepts of serum pathology.

SUMMARY.

1. Normal goat serum in which the complement has been completely destroyed by heat is capable of exerting considerable influence on hemolytic action, when tested with a goat serum immunized against sheep corpuscles.

2. The action of the third serum component or the substance remaining after such heating, varies greatly in different experiments. In one experiment it may be purely auxilytic; in another, purely antilytic; in a third, auxilytic in certain amounts and antilytic in others; and in a fourth, practically inactive.

3. Sera of different normal animals, heated under identical conditions, may differ widely in the action of their third components.

4. Differences in the length of heating and in the temperature at which it is heated, produce marked differences in the third component of the same normal serum.

5. Normal serum, heated in bulk to 56° C., loses its complement in about 20 minutes. On prolonging the heating there are

formed in the order in which they are mentioned: (1) a primary auxilysin, (2) a primary antilysin, (3) a secondary auxilysin, and (4) a tertiary auxilysin. Each of these substances appears, and afterward in part or wholly disappears, at a definite time during the heating.

6. When heated to 59° C., the same general phenomenon takes place, except that the events occur with greater rapidity.

7. Different normal sera, heated under identical conditions, differ but slightly in the times at which the various antilytic and auxilytic substances are produced and destroyed. They differ, however, greatly, in the amount of each substance so formed.

8. At no time during prolonged heating, does the third component acquire independent hemolytic powers, and at no time does it become capable of giving hemolytic properties to pure component or to pure amboceptor.

9. No theories are as yet advanced as to the nature of the auxilytic and antilytic substances herein reported, nor as to the bearing of the above facts on fundamental theories of serum pathology.